

Behavioral Regulation of Hemolymph Osmolarity Through Selective Drinking in Land Crabs, *Birgus latro* and *Gecarcoidea lalandii*

CHRISTIAN A. COMBS, NICOLE ALFORD, ANGELA BOYNTON,
MARK DVORNAK, AND RAYMOND P. HENRY

Department of Zoology and Wildlife Science, 101 Cary Hall, Auburn University, Alabama 36849

Abstract. Drinking behavior in *Birgus latro* and *Gecarcoidea lalandii* was videotaped under controlled laboratory conditions. *B. latro* displayed the drinking behavior typically observed in nature, spooning up water with the chelae (Lister, 1888; Gross, 1955). *G. lalandii* is documented for the first time displaying this same behavior; however it obtained water primarily through immersion. Under normal hydrated conditions (hemolymph osmolarities < 1050 mOsm) *B. latro* showed no preference for drinking fresh or seawater. When dehydrated (hemolymph osmolarities > 1050 mOsm) *B. latro* altered its drinking behavior and showed a distinct preference for freshwater. This strategy resulted in restoration of original hemolymph osmolarities and wet weights and was accomplished through periods of intensive drinking activity. Conversely, *G. lalandii* never experienced true dehydration; rather, the hemolymph became hyperosmotic compared with control animals. This species preferred freshwater both under normal and hemoconcentrated conditions. *G. lalandii* was also able to osmoregulate behaviorally and was able to restore hemolymph osmolarities to normal concentrations via immersion in freshwater following experimentally induced hemoconcentration. Possible physiological and ecological reasons for the differences in water uptake strategies and preferences are discussed.

Introduction

The transition from the aquatic to the terrestrial environment was both problematic and beneficial for crabs. Although oxygen was more readily available and there were new resources to exploit, certain morphological,

physiological, and behavioral strategies were required to overcome the barrier of desiccation and concomitant increase in hemolymph ion concentrations. Changes in hemolymph ion concentrations can profoundly affect many physiological processes in crustaceans including respiration, acid-base status, intracellular fluid volume, nitrogenous waste elimination, and enzyme function (Harris, 1977; Burrigren and McMahon 1981, 1988; Morris *et al.*, 1988; Wheatly *et al.*, 1984; Wood *et al.*, 1986; for reviews see Huggins and Munday, 1968; Schoffeniels, 1976; Gilles and Pequeux, 1981; Mangum, 1981; Taylor, 1982; Yancey *et al.*, 1982). In land crabs, dehydration and changes in hemolymph concentration are resisted using combinations of adaptations such as immersion, burrowing, water storage in the body or branchial chambers, evolutionary reduction in gill size, urine reprocessing, excretion of nitrogenous waste as urea or uric acid, and drinking (Bliss and Mantel, 1968; Bliss, 1979; Mantel and Farmer, 1983; Powers and Bliss, 1983; Wolcott and Wolcott, 1985, 1991; Greenaway, 1988; Greenaway *et al.*, 1988; T. G. Wolcott, 1988; D. L. Wolcott, 1991).

Drinking, or spooning up water with the chelae to the mouthparts, has been documented in *Gecarcoidea natalis*, *Geograpsus grayi*, *Cardisoma guanhumi* (Gross *et al.*, 1966), *C. carnifex* (Greenaway, 1988), *Gecarcoidea lalandii* (this paper), and in *Birgus latro* (Lister, 1888). Fresh and seawater are available to most land crabs, but it is not known precisely how the more terrestrial species use the two to regulate the concentration of their hemolymph. These crabs inhabit islands in the Indo-Pacific region where rainfall is seasonal and water sources other than the ocean may become scarce at certain times of year (Gross, 1964). This study examines the drinking preference of two of the more strictly terrestrial crabs, *Birgus*

latro (Anomura) and *Gecarcoidea lalandii* (Brachyura), when hemolymph concentrations are normal and hemoconcentrated (with possible dehydration).

Materials and Methods

Animal collection and maintenance

Specimens of both species, *Birgus latro* (500–2100 g) and *Gecarcoidea lalandii* (65–220 g) were obtained from the islands of Palau and Pohnpei and were shipped via air-freight in damp burlap inside coolers or ice chests. They were maintained in isolated Nalgene tanks (*B. latro*) or fabricated wooden pens with plexiglass partitions (*G. lalandii*) in a dark room at approximately 25°C. All were fed coconut, lettuce, and apples every other day, and were given either dechlorinated tapwater or seawater (35–40 ppt, as determined with a refractometer) depending on the subsequent testing regime.

Protocol

Observation chambers were constructed using standard 75 and 115 l aquaria fitted with a false bottom of plexiglass to facilitate viewing and minimize water spillage. Two circular holes were cut side-by-side in the false bottom to allow access to a pair of glass preparation dishes (115 × 50 mm, Fisher) that were secured with silicone to the aquarium floor. The tops of the dishes were flush with the plexiglass platform, simulating pools of water. The chamber was darkened on three sides to isolate each crab. The uncovered end of the aquaria permitted head-on viewing of both bowls. A plexiglass cover with air holes for ventilation was secured to each tank to prevent escape.

Drinking behavior of animals was recorded for individuals displaying hemolymph osmotic concentrations typically found for crabs sampled in the field (<1050 mOsm) (as reported by Henry and Cameron, 1981; Greenaway, 1988) and after hemoconcentration with possible dehydration (hemolymph osmolarity > 1050 mOsm). The former condition was maintained by allowing crabs to drink ad libitum from both fresh and seawater. Hemoconcentration was achieved through a combination of water deprivation and allowing access to only hypersaline water (>35 ppt). Animals were weighed daily and were not allowed to lose more than 12% of their initial wet weight, a value that was within the maximal tolerable levels of dehydration as reported by Kormanik and Harris (1981) and Burggren and McMahon (1981). Hemolymph osmolality was measured concurrently to determine at what point values exceeded 1050 mOsm, after which an experiment was begun.

Individual crabs were randomly assigned their initial condition, normal or hemoconcentrated. Behavior of each individual was recorded under both normal and hemo-

concentrated conditions, allowing at least a 48 h recovery period between experiments.

All crabs were allowed to adjust to the chambers for 24 h prior to observation/videotaping, while remaining on their water regime. Immediately preceding an observation period, crabs were weighed to the nearest 0.1 g on a top loading balance (Sartorius), and a blood sample (0.1 ml) was taken from the infrabranial sinus. The water in each chamber was replaced with 225 ml of seawater (35 ppt) and 225 ml of fresh (deionized) water was placed randomly in either the right or left dish. The crabs were then recorded for 12 h (1800–0600 h) using a Panasonic VHS Recorder Model AG-HT3. After 12 h, the crabs were weighed, blood samples were drawn, and the water volume remaining in each bowl was measured to the nearest 1.0 ml. Evaporative water loss was quantified using duplicate bowls of freshwater and seawater placed in an empty chamber. Temperature and relative humidity were also recorded using a ExTech Instruments Digital Humidity/Temperature Meter.

Hemolymph samples were placed in microcentrifuge tubes and kept on ice. After being sonicated at 20 watts for 10 s with a Microson Cell Disruptor CM-1 convertor (Heat Systems—Ultrasonics, Inc.), the samples were centrifuged for 1 min using a Micro-Centrifuge Model 235B (Fisher). Osmolarity was determined on 10 μ l samples of serum using a Wescor 5100C Vapor Pressure Osmometer.

Quantification of the number of drinks, time spent drinking, and time spent immersed in the water bowl were determined from video tape analysis. An individual drink was considered to be a cheliped sweep from the water to the mouth and subsequent sweep by the maxillae over the cheliped to remove the water (Lister, 1888). Time spent drinking was designated as the time of the first cheliped sweep to the time of the last cheliped sweep. Immersion time was considered to be the amount of time that a crab had part of its carapace submerged in a water bowl.

Statistics

Paired *t*-tests were used to determine if there were differences between water preferences within hemolymph concentration treatments, weight differences between hemolymph concentrations, and starting and ending hemolymph concentrations between treatments. Analysis of variance (ANOVA) was used to determine if there were differences between the preferences of crabs for drinking freshwater or seawater between hemolymph concentration conditions in each species. To reduce biases inherent in individual animals, only individuals that were tested at both hemolymph conditions were included in the statistical analysis. Scheffe's multiple comparisons test was used to compare means of the variables between normal and

hemoconcentrated treatments. All data were tested for normality using the Wilkes-Shapiro test and can be assumed to be normally distributed unless specified. All statistical analyses were accomplished with the SASTM statistical computer package (SAS Inst., Inc., 1982).

Results

Strategies of water uptake

The two species employed different strategies of water uptake both to maintain a normal hemolymph osmotic condition and to reduce hemolymph concentrations in response to hemoconcentration (often accompanied by dehydration in *B. latro*). *B. latro* used cheliped drinking as the only means of water uptake, spending 100% of their drinking time in that behavior; immersion in the water bowls was not employed, although the bowls could have accommodated at least portions of their bodies. The observed drinking behavior was virtually identical to that reported previously by Lister (1888). *G. lalandii*, however, was observed in cheliped drinking behavior only 2% of the time; the remainder of the time in contact with water was spent with all or part of the carapace immersed in the water bowl. When this species did engage in cheliped drinking, the behavioral pattern was similar to that seen in *B. latro*.

Normal water uptake and response to hemoconcentration

All specimens of *B. latro* that began an experiment in a normal hemolymph concentration state (848 ± 22 mOsm) were able to maintain that state over the 12 h observation period (t -test: $P > 0.81$) (Fig. 1). Under normal conditions, *B. latro* spent an average of 61 min engaged in drinking, performing 660 individual cycles of cheliped sweeps during a 12-h experiment. This species showed no preference for fresh or seawater, either with respect to the percent of the total drinks taken from each bowl (t -test: $P > 0.73$), the time spent drinking from each bowl (t -test: $P > 0.2$), or the percent of volume that was drunk (t -test: $P > 0.2$) (Fig. 2).

When individuals were hemoconcentrated prior to an experiment (1171 ± 51 mOsm), both the overall drinking behavior and the drinking preference were altered. Animals in a hemoconcentrated condition increased their total drinking time by over four-fold to 273 min, taking an average of 2702 individual drinks during that time. These animals displayed a distinct preference for freshwater for all three variables measured: total drinks, time of drinking, and volume consumed (t -test: $P < 0.01$) (Fig. 2). In addition, the drinking preferences were different between the two osmotic states (ANOVA: $P < 0.01$) with all control crabs exhibiting the same preference and all hemocon-

centrated crabs exhibiting the same preference (Scheffe: $P < 0.05$). This behavior led to a significant difference in osmolarity changes before and after the 12-h tests between the normal and hemoconcentrated treatments (t -test: $P < 0.02$), with the hemoconcentrated animals reducing their average osmolarity by 18% to 948 ± 26 mOsm. In addition, differences in weight before and after the 12-h tests were significantly different between control and hemoconcentrated animals (t -test: $P < 0.01$) with normal animals averaging only a 5 ± 12 g weight gain while hemoconcentrated animals gained 75 ± 16 g (Fig. 1). Therefore, *B. latro* compensated for hemoconcentration via water gain, indicating that initial hemoconcentration was accompanied by dehydration.

All specimens of *Gecarcoidea lalandii* that began an experiment in a normal hemolymph concentration state (947 ± 31 mOsm) were also able to maintain that state over the 12-h observation period (t -test: $P > 0.77$) (Fig. 3). On average, specimens of *G. lalandii* that began an experiment in a hemoconcentrated state (1168 ± 27 mOsm) were able to reduce their hemolymph concentration back to control levels (t -test: $P > 0.30$) over the 12-h observation period. Freshwater was significantly preferred over seawater at both osmotic states (t -test: $P < 0.05$ and $P < 0.1$, respectively) when immersion time was used as an indicator of behavioral osmoregulation (Fig. 4). Moreover, there is a significant difference in the amount of time spent immersed in seawater between the two osmotic states, with more time being spent in seawater when individuals started the experiments hemoconcentrated (ANOVA: $P < 0.05$, Scheffe: $P < 0.05$). The data for number of cheliped drinks and time spent cheliped drinking were not normally distributed, but freshwater was overwhelmingly preferred at both hydration states (Fig. 4) for the small amount of time, 2%, this species spent cheliped drinking. End-volume differences in the drinking bowls were not examined in this species due to the propensity of the animals to splash water out of the bowls while entering and exiting from them. This obscured any differences that might have been due to their drinking or absorbing water. Differences in weight before and after the 12-h tests were not significantly different between control and hemoconcentrated animals (t -test: $P > 0.25$). Therefore, *G. lalandii* did not compensate for hemoconcentration by water gain but rather appeared to reduce hemolymph ion concentrations through ion exchange via immersion in the ambient water.

Discussion

Both *Birgus latro* and *Gecarcoidea lalandii* can osmoregulate behaviorally, by selecting drinking water of the appropriate salinity, under laboratory conditions. Using this strategy they were able to maintain hemolymph

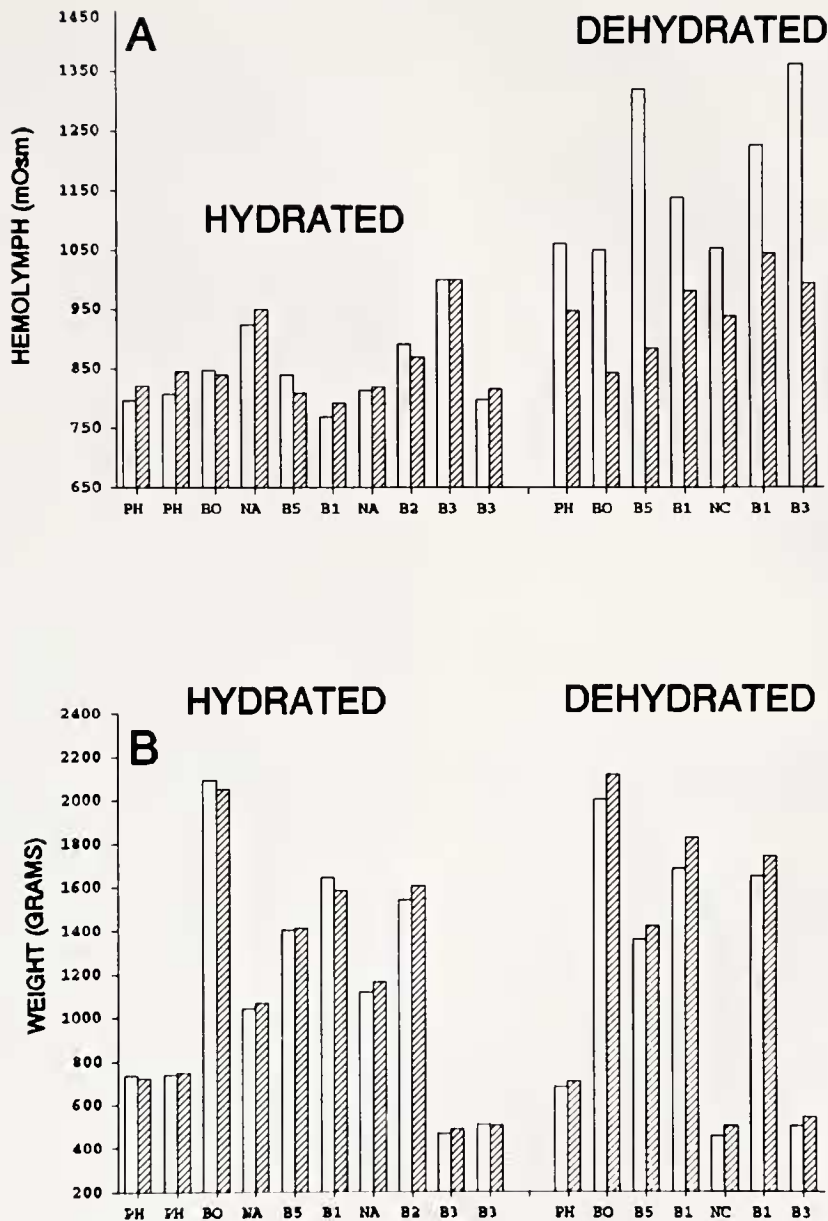


Figure 1. (A) Hemolymph concentrations (mOsm), and (B) weight (g), before (open bars) and after (shaded bars) 12-hour tests involving *Birgus latro*. Left-hand regions of figure are tests with initially normal hemolymph concentrations (<1050 mOsm) and right-hand regions are tests with initially concentrated hemolymph osmolarities (>1050 mOsm). X-axis labels identify individual specimens.

concentration and wet weight at normal hydrated levels during 12-h test periods. When hemoconcentrated, both species could reduce their hemolymph osmolarities to normal levels. This was accomplished by ion exchange in *G. lalandii*, whereas *B. latro* took on water to dilute the hemolymph.

Gross (1955) first documented the ability of *B. latro* to osmoregulate behaviorally. The present study indicated somewhat different results for drinking preference than did Gross (1955). In our study, specimens of *B. latro* at

normal hemolymph concentrations (hypoosmotic to seawater) showed no preference for either fresh or seawater; rather, they precisely regulated their hemolymph osmolarity through piecemeal drinking of both water types. In contrast, Gross (1955) reported that hydrated crabs preferred freshwater, but did drink some seawater. Our results coincide with those of Gross (1955) in that dehydrated crabs (hemolymph concentrations hyperosmotic to seawater) preferred freshwater. The difference in results between the two studies may be a result of the manner by

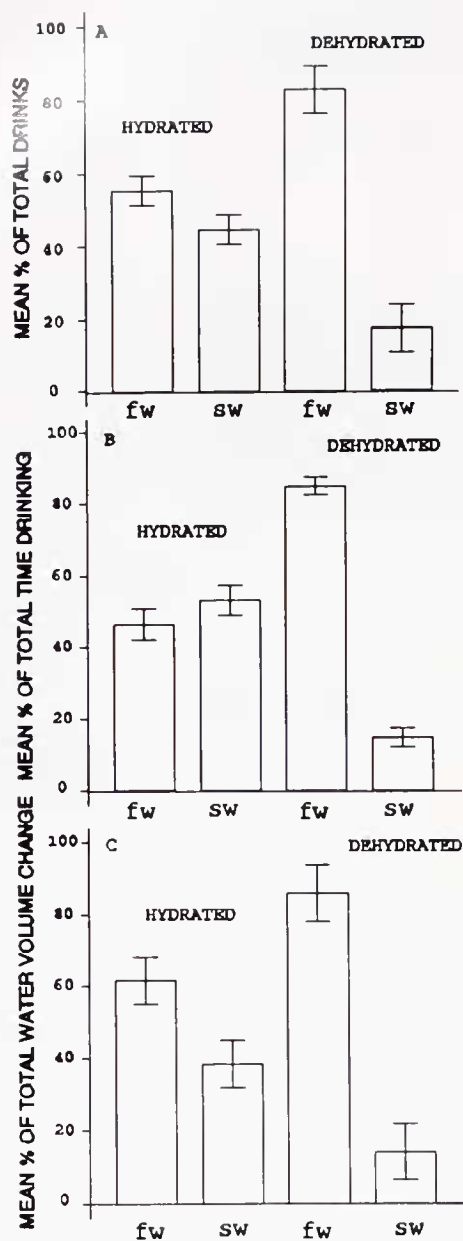


Figure 2. Drinking preference for freshwater or seawater according to initial hemolymph concentration (hydrated and dehydrated) during 12-hour tests involving *Birgus latro* ($n = 5$). Drinking preference is expressed as (A) mean percentage of total drinks, (B) mean percentage of total time spent drinking, (C) mean percentage of total volume change from water bowls (normalized for evaporative water loss).

which the quantification of the drinking preference was obtained. Gross (1955) quantified drinking behavior indirectly. Drinking behavior was monitored by etchings made on Kymograph drums caused by depressions of platforms over drinking bowls (see Gross, 1955, 1957 and Gross and Holland, 1960 for details). These etchings were then used to quantify drinking bouts. Precautions were taken in his study to reduce recording of behavior other

than drinking, but because these recordings were unattended it is unclear whether all the behavior recorded was actually drinking. Our study had the distinct advantage of directly viewing all of the animals' behavior, thereby allowing differentiation of drinking and exploratory behaviors, which enabled precise quantification of drinking behavior.

Birgus latro usually inhabits sand burrows or piles of decaying vegetation during the day and forages at night when ambient temperatures are cooler (Gross, 1964). These crabs employ both physiological and behavioral means of osmoregulation, although the main method appears to be behavioral avoidance of desiccation (Gross, 1964) along with uptake of water by drinking from inland pools. *Birgus latro* also uses a suite of physiological adaptations for osmoregulation. *B. latro* can reabsorb salts from the urine (Harris and Kormanik, 1981; Greenaway and Morris, 1989) and it has evolved the ability to excrete uric acid and therefore waste less water in nitrogen elimination (Bliss and Mantel, 1968; Kormanik and Harris, 1981; Greenaway and Morris, 1989). During periods of dehydration, specimens of *B. latro* continue to produce isotonic urine and maintain intracellular fluid volume while sacrificing extracellular stores (Burggren and McMahon, 1981; Harris and Kormanik, 1981). The large abdomen is the water storage site in *B. latro* (Harris and Kormanik, 1981) and becomes quite distended when fully hydrated, but only a small volume of water is stored in the branchial cavity relative to other terrestrial crabs (Wood and Boutilier, 1985). In addition, the gills are highly reduced (Cameron, 1981), thus limiting evaporative water loss, and they are used as exchange sites for ions, water, and carbon dioxide with oxygen uptake taking place at the primitive lung (Greenaway *et al.*, 1988). Thus, it seems that *B. latro*'s ability to differentiate between water of different salinities and its precise regulation of hemolymph concentration through piecemeal drinking augment its suite of other behavioral and physiological mechanisms and help to explain its high degree of terrestriality.

Birgus latro has never been observed drinking seawater directly from the ocean, although tracks have been found on dunes close to the shoreline (Gross, 1964), and Grubb (1971) reported anecdotal evidence of coconut crabs visiting the ocean. Considering the evidence presented in this study as well as in Gross (1955), *B. latro* might use the ocean as a water source under certain conditions. Field studies investigating the natural behavior of these crabs, particularly on some of the dry Pacific atolls, would help bring the findings of this study into context with the natural strategies these animals employ to osmoregulate behaviorally.

Gecarcoidea lalandii usually inhabits dry inland burrows (Bliss, 1968) and probably relies on intermittent access to dew and rain, along with soil water, for water up-

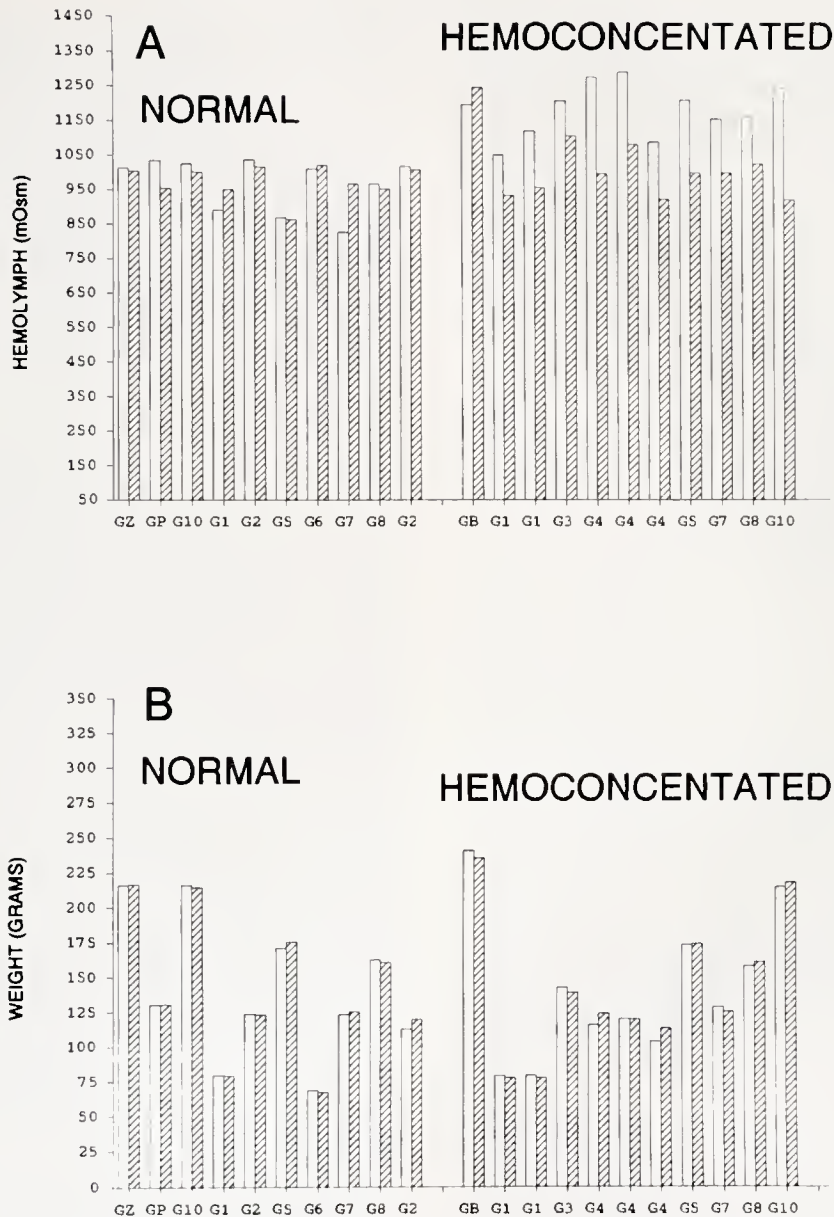


Figure 3. (A) Hemolymph concentrations (mOsm), and (B) weight (g), before (open bars) and after (shaded bars) 12-hour tests involving *Gecarcoidea landii*. Left-hand regions of figure are tests with initially normal hemolymph concentrations (<1050 mOsm) and right-hand regions are tests with initially concentrated hemolymph osmolarities (>1050 mOsm). X-axis labels identify individual specimens.

take (Wolcott, 1988). Although it is a highly terrestrial crab, its gills are not as reduced as those of *B. latro* (Cameron, 1981) and are therefore more subject to evaporative water loss. In this study, specimens of *G. landii* preferred to immerse themselves in, and drink from, freshwater regardless of initial blood condition. This contrasts with the water uptake strategy employed by their congener *G. natalis*, which prefers to drink (Gibson-Hill, 1947). This is the first quantification of drinking preference for this species. The preference for freshwater is not surprising con-

sidering the recent work of Wolcott and Wolcott (1985, 1991) who showed that other brachyurans (*Gecarcinus lateralis* and *Ocypode quadrata*) can reabsorb salts through urine reprocessing in the branchial chamber. Perhaps this and other physiological and behavioral osmoregulatory strategies explain why brachyurans do not need to rely heavily on seawater for their water budget. Wolcott and Wolcott (1988) conclude that *G. lateralis* inhabiting the island of Bermuda seldom if ever comes in contact with seawater except when spawning. Further, they conclude

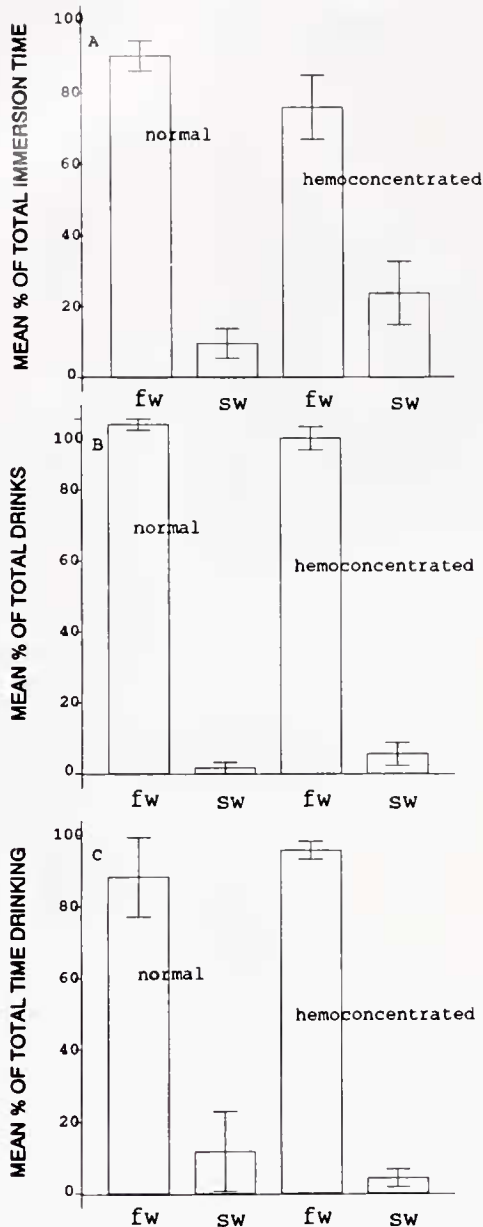


Figure 4. Drinking preference for freshwater or seawater according to initial hemolymph concentration (hydrated and dehydrated) during 12-hour tests for *Gecarcoidea lalandii* ($n = 5$). Drinking preference is expressed as (A) mean percentage of total immersion time, (B) mean percentage of total number of cheliped drinks, (C) mean percentage of total time spent cheliped drinking.

that most of the drinking water available to land crabs on Bermuda is freshwater, even near the edge of the shore. Brachyurans living on some islands in the Indo-Pacific region however, face seasonal paucity of freshwater (Gross 1964), but their behavior during these times has yet to be reported.

Land crabs in general are euryhaline (Mantel and Farmer, 1983) and can withstand a wide range of he-

molymph concentrations. Restoration of hemolymph osmolarities to normal levels after dehydration occurs quickly in both species but by different methods. This phenomenon may be a result of the natural unavailability of water sources (other than the ocean) at certain times of year thus forcing animals to rehydrate quickly after desiccation whenever favorable situations occur. This is evidenced by the amazing amount of time (5 h av.) and activity (>2500 individual cheliped drinking cycles) expended by *B. latro* in rehydrating itself after desiccation. In contrast, *B. latro* spent an average of only 1 h per night drinking, performing only about 500 individual cheliped drinking cycles when hydrated.

G. lalandii preferred to immerse itself rather than drink in order to obtain water, although it did occasionally drink. The reason for this difference in strategy is unclear, but it could have both a physiological and ecological basis. *B. latro* experienced both hemoconcentration and dehydration (*i.e.*, weight loss) when deprived of water. This suggests that some tissue water loss occurs, most probably from the large abdomen, in addition to a reduction in hemolymph volume. Therefore, it is possible that *B. latro* drinks to replenish tissue water content. *G. lalandii*, however, appears only to undergo hemoconcentration, and this may be related to the fact that this species lacks an obvious store of tissue water. As a consequence, hemolymph volume may be sacrificed to maintain tissue water content, and the major function of immersion may be to alleviate a hemolymph ion load, which can be done readily across the gills. Thus, the different strategies of rehydration may be in answer to two different physiological stresses.

The decrease in time devoted to obtaining water via immersion *versus* cheliped sweeps (2.5 min average following desiccation and 1.3 min average when hydrated per 12-h test session) may also decrease the risk of predation in *G. lalandii*, which is smaller and potentially more vulnerable than *B. latro*.

Further research involving behavioral osmoregulation may investigate the feedback mechanisms of internal and external osmoreceptors. Internal receptors that monitor blood osmolality have not yet been found. However, in other species, hormones produced by cells in the optic ganglia, brain, and thoracic ganglia affect the movement of salts and water across the gills, renal organ membranes, and gastrointestinal wall (Hill and Wyse, 1989). Greenaway (1988) speculated that *B. latro* has osmoreceptors on the chelae or mouth parts. It is quite possible that *B. latro* and *G. lalandii* also have internal blood osmotic receptors and that feedback mechanisms involving hormones and external osmoreceptors enable it to choose water of the appropriate salinity to maintain normal blood osmolalities. Future work involving manipulation of blood osmolality and testing with the video protocol we have established might initiate understanding of the

mechanisms that enable *B. latro* and *G. lalandii* to osmoregulate behaviorally.

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